

Scientific paper

# Speciation of Ternary Complexes of Some Essential Metal Ions with L-Glutamic Acid and L-Methionine in 1, 2-Propanediol – Water Mixtures

Muddapu Padma Latha, Vegi Maheswara Rao, Tirukkavalluri Siva Rao and Gollapalli Nageswara Rao\*

School of Chemistry, Andhra University, Visakhapatnam-530 003, India

\* Corresponding author: E-mail: gollapallinr@yahoo.com

Received: 11-05-2007

## Abstract

Chemical speciation of ternary complexes of Co(II), Ni(II), Cu(II), and Zn(II) ions with L-glutamic acid and L-methionine have been studied pH metrically in the concentration range of 0–60% volume/volume 1,2-propanediol-water mixtures maintaining an ionic strength of 0.16 mol dm<sup>-3</sup> at 303 K. Alkalimetric titrations were carried out in different relative concentrations (M: L: X = 1:2:2, 1:2:4, 1:4:2) of metal (M) to L-glutamic acid (L) to L-methionine (X). Stability constants of ternary complexes were calculated and various models were refined with MINIQUAD75. The best-fit chemical models were selected based on statistical parameters and residual analysis. The species detected are MLX and MLX<sub>2</sub> for Co(II); MLX, ML<sub>2</sub>X and MLXH for Ni(II); MLX, MLXH and MLXH<sub>2</sub> for Cu(II); MLX and MLXH for Zn(II). The chemical speciation, metal bioavailability and transportation are explained based on the stability constants.

**Keywords:** Chemical speciation; ternary complexes; L-glutamic acid; L-methionine; 1,2-propanediol

## 1. Introduction

L-Glutamic acid (Glu) plays a vital role in various biochemical processes at the molecular levels. In the animal world, it links the metabolism of carbon and nitrogen. In the central nervous system (CNS) glutamate has two functions: it is a neurotransmitter and a precursor of  $\gamma$ -aminobutyric acid.<sup>1,2</sup> It is the main excitatory transmitter of the CNS of vertebrates,<sup>3</sup> being involved in fundamental mechanisms of learning, memory, formation, and plasticity of the synaptic endings and in the development of the CNS. In many animals Glu is the most abundant intracellular amino acid.

L-Methionine (Met) is an essential amino acid and its dietary requirement is about 2 gm/day.<sup>4</sup> Mammalian tissues (other than humans) and many bacteria synthesize it from cobalamine – enzyme where as all the plants and some bacteria synthesize it from homocysteine.<sup>5</sup> Met is synthesized from cysteine and o-phosphohomoserine involving three enzymes,<sup>6</sup> cystathionine  $\gamma$ -synthase, cystathionine  $\beta$ -lyase and methionine synthase. Met acts as a methyl group donor in biological transmethylation processes in the form of S-adenosyl methionine.<sup>7</sup> The proto-

nation constants of Glu and Met,<sup>8</sup> their binary complexes with Pb(II), Cd(II), Hg(II), Co(II), Ni(II), Cu(II) and Zn(II) and ternary complexes with Pb(II), Cd(II) and Hg(II) in 1,2-propanediol (PG)-water mixtures were reported earlier.<sup>9–11</sup> The purpose of using the co-solvents is to mimic the active site cavities of the biological systems. Previously we studied the speciation of different systems in DMF, DMSO and urea-water mixtures. In the present study we have chosen propylene glycol as co-solvent to study the effect of decreasing dielectric constant of the medium on the stability constants of the complexes.

The mixed ligand complexes of  $\beta$ -picoline, lysine and methionine with lead have been studied in aqueous medium at constant ionic strength.<sup>12</sup> The ternary complexes containing Glu have also been studied.<sup>13–15</sup> Mixed ligand complexes of Cr(II) with Met and Glu and Cd(II) with Glu, formic acid and ornithine have been reported in the literature.<sup>16,17</sup>

Based on the importance of Glu and Met and their involvement in various physiological reactions, speciation studies of their ternary complexes with some essential metal ions like Co(II), Ni(II), Cu(II) and Zn(II) in PG – water mixtures have been undertaken. A trace element is considered as essential if it meets the following criteria: a)

It is present in all healthy tissues. b) Its concentration from one species to the next is fairly constant. c) Depending on the species studied, the amount of each element has to be maintained within its required limit if the functional and structural integrity of the tissues is to be safeguarded, and the growth, health, and fertility to remain flawless. d) Its withdrawal induces reproducible physiological and structural abnormalities. e) Its addition to the diet either prevents, or reverses, the abnormalities.<sup>18</sup>

## 2. Experimental

Aqueous solutions (0.1 mol dm<sup>-3</sup>) of Co(II), Ni(II), Cu(II) and Zn(II) chlorides (G.R Grade, E-Merck, Germany) were prepared by dissolving them in triple distilled water 0.05 mol dm<sup>-3</sup> aqueous solutions of Glu (E-Merck, Germany) and Met (Himedia, India) were also prepared. To increase the solubility of the ligands, 0.05 mol dm<sup>-3</sup> nitric acid was maintained in the solutions. PG (Merck, India) was used as received. The strength of alkali was determined using the Gran plot method.<sup>19</sup> Errors in the concentrations of ligand, metal ions and alkali were subjected to analysis of variance (ANOVA).<sup>20</sup>

The titrations were carried out in the medium containing varying concentrations of the PG maintaining an ionic strength of 0.16 mol dm<sup>-3</sup> with sodium nitrate at 303.0±0.1 K. The measurements were recorded with an

ELICO (Model LI-120) pH meter of 0.01 readability in conjunction with a glass and calomel electrode. The pH meter was calibrated with 0.05 mol dm<sup>-3</sup> potassium hydrogen phthalate in acidic region and 0.01 mol dm<sup>-3</sup> borax solution in basic region. The glass electrode was equilibrated in a well stirred PG-water mixture containing inert electrolyte. The effect of variations in asymmetry potential, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode are accounted for in the form of correction factor (log F) which was computed from the experimental and simulated acid-base titration data calculated by SCPHD program.<sup>21,22</sup> A correction was applied to the pH meter dial readings to account for the solvent effect on pH.

Titration of strong acid with alkali was carried out at regular intervals to check whether complete equilibrium was achieved. The calomel electrode was refilled with PG-water mixture of equivalent composition as that of the titrand. In each of the titrations, the titrand consisted of 1 mmol of nitric acid in a total volume of 50 cm<sup>3</sup>. Titrations were carried out in the presence of different relative concentrations of the metal (M) to Glu (L) and Met (X) (M:L:X = 1:2:2, 1:2:4, 1:4:2) with 0.4 mol dm<sup>-3</sup> NaOH. The analytical concentrations of the ingredients are given in Table 1. The details of experimental procedure and titration assembly are given elsewhere.<sup>23</sup>

The best-fit chemical model for each system investigated was arrived at using non-linear squares analysis

**Table 1:** Total initial concentrations of ingredients (in mmol) for mixed-ligand titrations in PG-water mixtures. [NaOH] = 0.4 mol dm<sup>-3</sup>; V<sub>0</sub> = 50.0 cm<sup>3</sup>; temperature = 303 K; ionic strength = 0.16 mol dm<sup>-3</sup>; nitric acid = 1 mmol.

% (v/v)	TMO				TLO		M:L:X
	Co(II)	Ni(II)	Cu(II)	Zn(II)	Glu	Met	
00.00	0.106	0.099	0.101	0.098	0.200	0.200	1:2:2
					0.200	0.400	1:2:4
					0.400	0.200	1:4:2
10.00	0.106	0.099	0.101	0.098	0.200	0.200	1:2:2
					0.400	0.200	1:2:4
					0.400	0.200	1:4:2
20.00	0.106	0.099	0.101	0.098	0.200	0.200	1:2:2
					0.400	0.200	1:2:4
					0.400	0.200	1:4:2
30.00	0.106	0.099	0.101	0.098	0.200	0.200	1:2:2
					0.400	0.200	1:2:4
					0.400	0.200	1:4:2
40.00	0.106	0.099	0.101	0.098	0.200	0.200	1:2:2
					0.400	0.200	1:2:4
					0.400	0.200	1:4:2
50.00	0.106	0.099	0.101	0.098	0.200	0.200	1:2:2
					0.400	0.200	1:2:4
					0.400	0.200	1:4:2
60.00	0.106	0.099	0.101	0.098	0.200	0.200	1:2:2
					0.400	0.200	1:2:4
					0.400	0.200	1:4:2

program MINIQUAD75,<sup>24</sup> which exploits the advantage of constrained least squares method in the initial refinement and reliable convergence of undamped, unconstrained Marquardt algorithm.

### 3. Results and Discussion

#### 3.1. Modeling of Chemical Speciation

A preliminary investigation of alkalimetric titrations of mixtures containing different mole ratios of Glu and Met in the presence of nitric acid and inert electrolyte inferred that no condensed species were formed. The protonation constants of the ligands and the stability constants of the binary metal complexes were fixed in the refinement of the stability constants of the ternary complexes and in testing various chemical models using MINIQUAD75. The best fit models were chosen based on the statistical parameters<sup>9</sup> like  $\chi^2$ , R-factor, skewness and kurtosis given in Table 2. The ternary complex species detected are MLX and MLX<sub>2</sub> for Co(II); MLX, ML<sub>2</sub>X and MLXH for Ni(II); MLX, MLXH and MLXH<sub>2</sub> for Cu(II); MLX and MLXH for Zn(II). The possibility of the existence of metal complexes of the form ML<sub>2</sub>XH<sub>n</sub> or MLX<sub>2</sub>H<sub>n</sub> were excluded because of the instability of the complexes due to the inability of the metal ions to accommodate three bulky ligands.

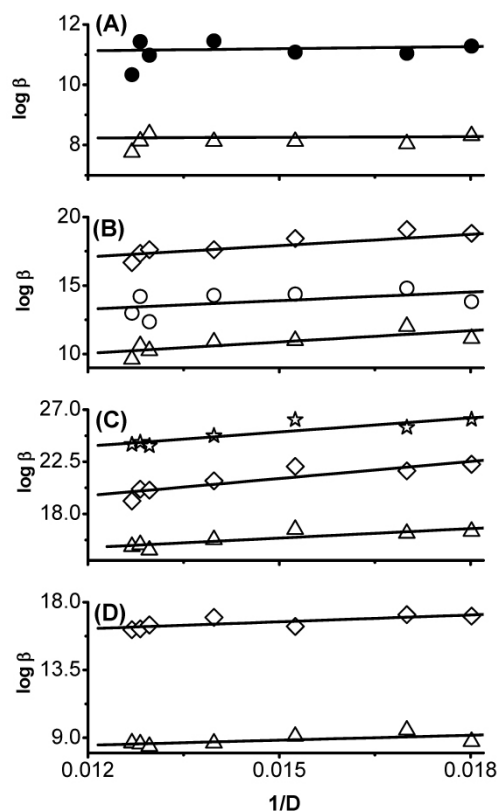
A very low standard deviation (SD) in log values of overall stability constants ( $\log \beta$ ) indicates the precision of these parameters. The small values of  $U_{\text{corr}}$  (the sum of the squares of deviations in the concentrations of the ligands and the hydrogen ion at all experimental points) corrected for degrees of freedom, indicate that the models represent the experimental data. Small values of mean, standard deviation and mean deviation for the systems corroborate that the residuals are around a zero mean with little dispersion. For an ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively. The kurtosis values in the present study indicate that most of the residuals are very nearer to mesokurtic and a few form leptokurtic patterns. The values of skewness recorded in Table 2 are between -1.68 and 0.87. These data evince that the residuals form a part of normal distribution; hence, least-squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R-values recorded.

#### 3.2. Effect of Dielectric Constant on the Stability of Ternary Complexes

PG is an amphiprotic solvent. It is a structure former and it enhances the water structure in PG-water mixtures; hence, it removes water from the coordination sphere of metal ions, making them more reactive towards the ligands. As a result, the stability of the complexes is expected to increase. At the same time, it is a coordinating sol-

vent, and it competes with the ligands for coordinating the metals. This decreases the stability of the complexes. Hence, the stability of the complexes is expected to either increase or decrease linearly. The variation of overall stability constants with co-solvent content depends upon electrostatic and non-electrostatic factors. Born's classical treatment holds good in accounting for the electrostatic contribution to the free energy change.<sup>25</sup> According to this treatment, the energy of electrostatic interaction is related to dielectric constant. Hence, the  $\log \beta$  values should vary linearly as a function of reciprocal of the dielectric constant ( $1/D$ ) of the medium, which is observed in the present study (Figure 1). The linear variation indicates that electrostatic forces are dominating the equilibrium process under the present experimental conditions.

The linear increase in the stability of the complexes indicates the dominance of structure forming nature of PG over its complexing ability. The cation stabilizing nature of cosolvents, specific solvent-water interactions, charge dispersion and specific interactions of cosolvent with solute (indicated by the changes in the solubility of different species in the aquo-organic mixtures) account for the little deviation of linear relationship.



**Figure 1:** Variation of magnitude of stability constants ( $\log \beta$  of ternary complexes of Glu-Met ŠRTF bookmark start:  $\text{cOLE\_LINK1}$  with reciprocal of constant ( $1/D$ ) in PG-water mixtures. ŠRTF bookmark end:  $\text{cOLE\_LINK1}$ ; (A) Co(II), (B) Ni(II), (C) Cu(II) and (D) Zn(II); ( $\Delta$ )  $\log \beta_{\text{MLX}}$ , ( $\circ$ )  $\log \beta_{\text{ML}_2\text{X}}$ , ( $\bullet$ )  $\log \beta_{\text{MLX}_2}$ , ( $\blacklozenge$ )  $\log \beta_{\text{MLXH}}$  and ( $\square$ )  $\log \beta_{\text{MLXH}_2}$ .

**Table 2.** Parameters of best fit chemical models of Co(II), Ni(II), Cu(II) and Zn(II)–Glu–Met complexes in PG–water mixtures.

% v/v PG	log $\beta$ (SD)					NP	$U_{\text{corr}}$	Skewness	$\chi^2$	R-Factor	Kurtosis	pH-Range
	MLX	ML <sub>2</sub> X	MLX <sub>2</sub>	MLXH	MLXH <sub>2</sub>							
<b>Co(II)</b>												
00.0	7.76(15)	–	10.33(12)	–	–	45	0.20	0.81	9.51	0.0078	3.97	4.0–9.3
10.0	8.13(27)	–	11.43(12)	–	–	45	2.49	0.27	11.29	0.0133	3.23	4.0–9.5
20.0	8.37(29)	–	10.98(29)	–	–	46	6.02	0.48	21.57	0.0211	3.54	4.0–9.5
30.0	8.12(10)	–	11.45(16)	–	–	52	3.17	–0.90	30.31	0.0149	3.07	4.0–10
40.0	8.12(23)	–	11.08(10)	–	–	46	5.26	0.43	17.74	0.0059	3.58	4.0–9.5
50.0	8.04(55)	–	11.04(14)	–	–	22	5.34	0.84	16.00	0.0076	5.15	7.5–9.5
60.0	8.31(40)	–	11.28(10)	–	–	21	0.72	–0.55	2.48	0.0091	3.80	8.0–10
<b>Ni(II)</b>												
00.0	9.62(28)	12.98(10)	–	16.67(36)	–	25	0.456	0.78	4.16	0.00647	4.52	6.0–9.0
10.0	10.62(22)	14.20(10)	–	17.34(34)	–	45	1.008	0.59	8.89	0.00849	4.10	4.0–9.5
20.0	10.25(10)	12.36(7)	–	17.62(8)	–	23	0.052	–0.23	8.70	0.00229	2.46	6.5–9.5
30.0	10.89(14)	14.28(8)	–	17.61(16)	–	46	0.057	0.38	16.35	0.00623	4.06	4.0–9.5
40.0	10.99(62)	14.37(14)	–	18.43(36)	–	22	0.637	–0.66	4.91	0.00790	3.42	6.5–9.5
50.0	12.02(27)	14.80(21)	–	19.09(27)	–	22	0.302	0.20	4.73	0.00550	3.13	6.5–9.5
60.0	11.14(14)	13.82(8)	–	18.81(12)	–	21	0.178	0.49	3.81	0.00408	3.72	6.5–9.5
<b>Cu(II)</b>												
00.0	15.18(8)	–	–	19.12(95)	23.99(14)	149	5.866	–1.68	182.0	0.00782	5.56	1.5–5.5
10.0	15.35(9)	–	–	20.14(14)	24.16(11)	56	0.376	–0.85	7.50	0.00353	4.95	2.5–6.0
20.0	14.88(19)	–	–	20.05(13)	23.87(31)	26	0.286	–0.01	13.23	0.0038	2.65	3.5–6.0
30.0	15.78(5)	–	–	20.85(4)	24.74(4)	57	0.184	–0.92	13.82	0.0024	6.33	2.5–6.0
40.0	16.67(34)	–	–	22.09(29)	26.12(31)	33	0.924	–0.92	2.79	0.00827	3.52	4.0–10
50.0	16.33(7)	–	–	21.71(4)	25.46(5)	62	1.008	–0.36	12.77	0.00172	4.90	2.5–6.0
60.0	16.50(13)	–	–	22.28(5)	26.15(6)	64	0.279	–0.06	6.06	0.00278	3.20	2.5–6.0
<b>Zn(II)</b>												
00.0	8.66(40)	–	–	16.17(63)	–	15	0.87	0.68	2.93	0.0080	3.72	7.0–8.8
10.0	8.59(39)	–	–	16.23(20)	–	19	0.357	0.37	3.58	0.00566	3.19	6.7–9.0
20.0	8.43(7)	–	–	16.49(21)	–	21	1.323	–0.13	2.67	0.01083	3.02	6.3–9.0
30.0	8.65(62)	–	–	16.99(9)	–	26	0.565	0.351	4.31	0.00757	3.97	6.8–9.5
40.0	9.10(17)	–	–	16.39(29)	–	19	0.362	0.26	5.68	0.00576	1.91	6.5–9.0
50.0	9.51(31)	–	–	17.17(6)	–	14	0.063	0.87	9.14	0.00248	6.11	7.0–9.0
60.0	8.76(31)	–	–	17.07(17)	–	24	0.855	–0.15	6.33	0.00907	2.83	6.5–9.5

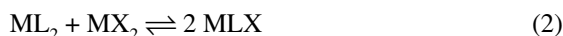
$U_{\text{corr}} = U/(NP-m) \times 10^8$ , where, m = number of species; NP = number of experimental points

### 3. 3. Quantification of Change in the Stability of Species

The change in the stability of the ternary complexes as compared to their binary analogues was quantified based on the disproportionation constant (log X) given by Equation 1.<sup>26–29</sup>

$$\log X = 2 \log K_{MLX}^M - \log K_{ML_2}^M - \log K_{MX_2}^M \quad (1)$$

which corresponds to the equilibrium



Under the equilibrium conditions one can expect the formation of 50% ternary complexes and 25% each of the binary complexes statistically and the value of log X shall

be 0.6.<sup>30</sup> A value greater than this, accounts for the extra stability of MLX.

Another approach to quantify the stability of ternary complexes was based on the difference in stability ( $\Delta \log K$ ) for the reactions ML with X and M<sub>(aq)</sub> with L and X,<sup>27,31</sup> where L is the primary ligand (Glu) and X is the secondary ligand (Met). It is compared with that calculated purely on statistical grounds as given in Equation 2.

$$\Delta \log K = \log K_{MLX}^M - \log K_{ML}^M - \log K_{MX}^M \quad (3)$$

The electrostatic theory of binary complex formation and statistical arguments suggest the availability of additional coordination positions of the hydrated metal ion for the first ligand than for the second. Hence, the usual order of stability  $K_{ML}^M > K_{MLX}^M$  applies. This suggests that  $\Delta \log K$  should be negative, although several exceptions have been found.<sup>32</sup> The statistical values of  $\Delta \log K$  for bi-

dentate L and X are  $-0.4$ ,  $-0.6$  and between  $-0.9$  and  $-0.3$  for octahedral, square planar and distorted octahedral complexes, respectively. Negative values of  $\Delta \log K$  can be understood as the secondary ligand forms a more stable complex with hydrated metal ion than with ML.

Whenever the experimental values of  $\Delta \log K$  exceed the statistical values, it can be inferred that the ternary complex is formed as a result of interaction of ML with X or MX with L.  $\Delta \log K$  values of ternary complexes containing bipyridyl as the primary ligand are positive for O-donors (malonic acid, pyrocatechol etc.),<sup>27</sup> negative for N-donors (ethylene diamine) and intermediate or negative for amino acids with both N and O coordination sites.<sup>33,34</sup> However, a very high negative value ( $-2.3$ ) for Cu(ethylene diamine)(iminodiacetic acid) and a positive value ( $0.82$ ) for Cu(o-phen)-(6,7-dihydroxynaphthalene-2-sulphonate) was also observed.

The  $\log X$  and  $\Delta \log K$  values calculated from binary and ternary complexes are included in Table 3. The equations for the calculation of  $\Delta \log K$  and  $\log X$  are given in chart 1. These values could not be calculated for some systems due to the absence of relevant binary species. In the present study, the  $\log X$  values range from  $-1.09$  to  $5.08$  and some values found to be higher than those expected on statistical bases ( $0.6$ ). These higher values account

for the extra stability of the ternary complexes.  $\Delta \log K$  values are in the range from  $-3.08$  to  $2.02$  which indicate that the ternary complexes formed by the Cu(II) are more stable compared to that of the other three metal ions. The reason for the extra stability of these complexes may be due to interactions outside the coordination sphere such as the formation of hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect and stacking interactions.<sup>35,36</sup>

### 3. 4. Effect of Influential Parameters on Stability Constants

Any variation in the parameters like concentrations of ingredients affects the magnitudes of equilibrium constants. Such parameters are called influential parameters. In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the concentrations of alkali, acid, ligands, metal and correction factor. The results of typical samples given in Table 4 emphasise that the errors in the concentrations of alkali and acid affect stability constant more than those of the ligands, metal and  $\log F$ .

**Table 3:** Variation of stability of ternary complexes of Glu-Met in PG-water mixtures.

% v/v PG	$\Delta \log K_{MLX}$	$\Delta \log K_{MLX2}$	$\log X_{MLX}$	$\Delta \log K_{MLX}$	$\Delta \log K_{ML2X}$	$\log X_{MLX}$	$\log X_{MLXH}$	
	<b>Co(II)</b>				<b>Ni(II)</b>			
00.00	-0.59	-1.61	0.41	-0.55	-1.61	-0.04	-	
10.00	-0.51	-0.82	0.58	-0.65	-1.36	0.61	0.76	
20.00	-0.20	-1.40	1.05	-0.66	-2.80	0.60	2.27	
30.00	-0.88	-1.41	-0.18	-0.50	-1.40	0.94	1.20	
40.00	-0.78	-1.86	-0.27	-1.29	-2.46	-0.08	1.88	
50.00	-1.39	-2.42	-1.09	0.05	-1.71	2.43	3.63	
60.00	-0.93	-2.12	-0.14	-0.75	-2.26	1.43	2.99	
	$\Delta \log K_{MLX}$	$\log X_{MLX}$	$\log X_{MLXH}$	$\Delta \log K_{MLX}$	$\Delta \log K_{MLXH}$	$\Delta \log K_{MLXH2}$	$\log X_{MLX}$	$\log X_{MLXH}$
	<b>Zn(II)</b>				<b>Cu(II)</b>			
00.00	-0.50	0.32	1.28	-0.52	-3.08	2.02	-	0.15
10.00	-0.59	-0.06	0.98	-1.03	0.93	0.36	0.93	1.31
20.00	-0.83	-0.35	1.73	-0.85	1.18	0.49	0.54	1.31
30.00	-0.71	-0.10	2.65	-0.67	1.37	0.66	1.79	2.30
40.00	-0.72	0.01	0.74	-	-	0.64	1.11	2.33
50.00	-0.74	0.22	2.01	-1.50	0.96	-0.23	0.41	1.35
60.00	-1.03	-0.05	1.83	-1.67	1.16	-0.01	0.96	1.86

**Chart 1:** Equations for the calculation of  $\Delta \log K$  and  $\log X$

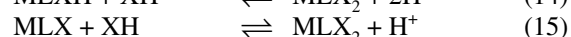
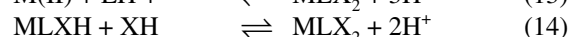
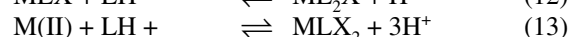
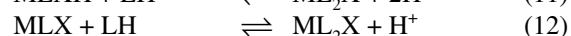
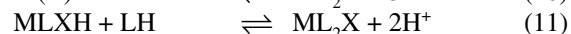
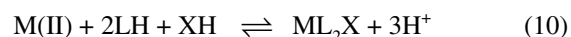
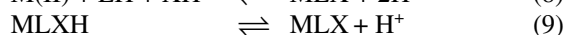
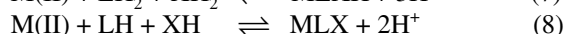
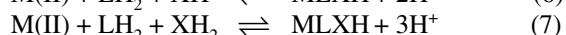
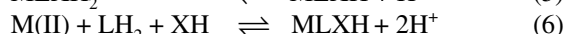
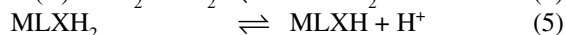
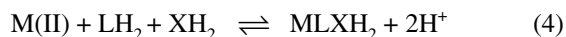
$$\begin{aligned}
 \Delta \log K_{1110} &= \log \beta_{1110} - \log \beta_{1100} - \log \beta_{1010} & \log X_{1110} &= 2 \log \beta_{1110} - \log \beta_{1200} - \log \beta_{1020} \\
 \Delta \log K_{1120} &= \log \beta_{1120} - \log \beta_{1100} - \log \beta_{1020} & \log X_{1111} &= 2 \log \beta_{1111} - \log \beta_{1202} - \log \beta_{1020} \\
 \Delta \log K_{1210} &= \log \beta_{1210} - \log \beta_{1200} - \log \beta_{1010} & &= 2 \log \beta_{1111} - \log \beta_{1200} - \log \beta_{1022} \\
 \Delta \log K_{1111} &= \log \beta_{1111} - \log \beta_{1100} - \log \beta_{1011} & &= 2 \log \beta_{1111} - \log \beta_{1201} - \log \beta_{1021} \\
 &= 2 \log \beta_{1111} - \log \beta_{1101} - \log \beta_{1010} \\
 \Delta \log K_{1112} &= \log \beta_{1112} - \log \beta_{1101} - \log \beta_{1011}
 \end{aligned}$$

**Table 4:** Effect of errors in influential parameters on the stability constants of ternary complexes of Ni(II) and Cu(II) with Glu-Met in 30% (v/v) PG-water mixture.

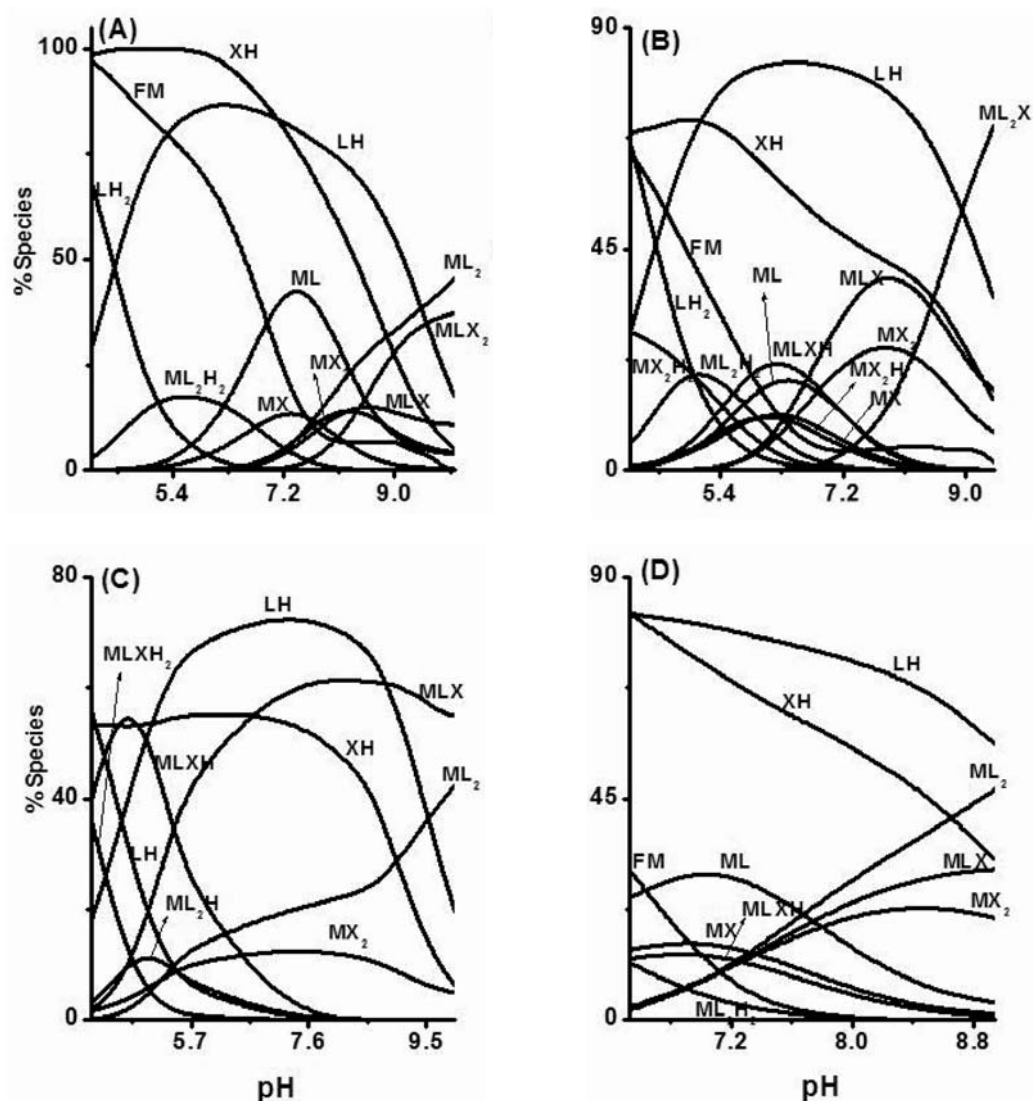
Ingredient	% Error	log $\beta$ (SD)					
		Ni(II)			Cu(II)		
		MLX	MLXH	ML <sub>2</sub> X	MLX	MLXH	MLXH <sub>2</sub>
	0	10.89(14)	17.61(16)	14.28(8)	15.78(5)	20.85(4)	24.74(4)
Alkali	-5	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected
	-2	Rejected	Rejected	Rejected	Rejected	Rejected	23.67(78)
	+2	12.53(13)	19.08(7)	16.98(16)	17.14(3)	21.61(3)	25.37(3)
	+5	Rejected	20.25(23)	20.27(32)	18.64(9)	22.36(11)	25.95(9)
Acid	-5	13.76(27)	20.08(13)	19.42(25)	18.64(7)	22.59(6)	26.12(7)
	-2	12.35(13)	18.96(7)	16.42(15)	17.08(3)	21.66(3)	25.45(3)
	+2	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected
	+5	Rejected	Rejected	Rejected	Rejected	Rejected	Rejected
Glu	-5	11.52(9)	18.22(7)	15.24(8)	16.03(3)	20.83(4)	24.64(5)
	-2	11.17(11)	17.90(10)	14.67(8)	15.89(4)	20.84(4)	24.70(4)
	+2	10.52(26)	17.08(50)	13.88(10)	15.66(6)	20.85(4)	24.77(4)
	+5	Rejected	Rejected	13.27(14)	15.41(8)	20.85(5)	24.83(4)
Met	-5	10.96(16)	17.67(18)	14.53(10)	15.75(4)	20.82(4)	24.64(5)
	-2	10.92(15)	17.64(17)	14.38(10)	15.77(4)	20.83(4)	24.70(4)
	+2	10.86(14)	17.59(16)	14.18(8)	15.79(5)	20.86(4)	24.77(4)
	+5	10.79(14)	17.55(16)	14.01(8)	15.81(5)	20.88(5)	24.83(4)
Metal	-5	11.13(12)	17.68(15)	14.65(8)	16.06(4)	20.97(4)	24.82(4)
	-2	10.99(13)	17.64(16)	14.43(8)	15.90(4)	20.89(4)	24.77(4)
	+2	10.77(16)	17.58(17)	14.14(9)	15.66(5)	20.79(4)	24.70(4)
	+5	10.55(22)	17.54(19)	13.39(9)	15.46(6)	20.71(4)	24.65(5)
Log F	-5	10.91(14)	17.65(16)	14.32(9)	15.85(5)	20.90(4)	24.82(4)
	-2	10.90(14)	17.63(16)	14.30(9)	15.81(5)	20.87(4)	24.77(4)
	+2	10.87(14)	17.57(18)	14.25(8)	15.75(5)	20.82(4)	24.70(4)
	+5	10.88(14)	17.60(17)	14.27(9)	15.70(5)	20.79(4)	24.65(4)

### 3. 5. Chemical Speciation

Distribution diagrams drawn using the formation constants of the best fit model are shown in Figure 2 which contain protonated and unprotonated species like MLXH, MLXH<sub>2</sub>, MLX, ML<sub>2</sub>X and MLX<sub>2</sub>. The distribution diagrams indicate the relative abundance of various forms of metal (chemical speciation) at different pH and dielectric conditions. A stable ternary complex shall be responsible for metal transportation in biological systems and the weak binary metal complexes make the essential metals bioavailable. The increased concentrations of complexing agents make the essential metals unavailable due to the formation of stable binary metal complexes. The formation of the complex species can be represented by the following equilibria. The charges of the species are omitted for clarity.



Depending upon the nature of the ligands and the metal ions and based on the basic chemical knowledge the structures of the ternary complexes were proposed as shown in Figure 3. These structures indicate that methionine acts as a bidentate ligand and glutamic acid acts as bidentate/tridentate ligand depending upon the pH conditions. In highly acidic medium the amino groups of both the amino acids are protonated and they do not participate in coordination as shown in the structure of MLXH<sub>2</sub>. In this species Met acts as monodentate and Glu acts as bidentate. In mono protonated ternary complexes like MLXH, Glu and Met act as bidentates and the proton is on Glu but not on Met. So, the species is M(LH)X but not M(L)(XH). In unprotonated species Glu acts as tridentate ligand, provided vacant coordination positions are available, as in MLX.



**Figure 2:** Species distribution diagrams of ternary complexes of Glu-Met; (A) Co(II), (B) Ni(II), (C) Cu(II) and (D) Zn(II) in 20% PG-water mixture.

## 4. Conclusions

A study of the speciation of ternary complexes of Co(II), Ni(II), Cu(II) and Zn(II) with Glu and Met in PG-water media reveals the compartmentalization of metabolic reactions. The following conclusions have been drawn from the modeling studies:

1. The ternary metal complex species detected are MLX, MLX<sub>2</sub> for Co(II), MLX, ML<sub>2</sub>X, MLXH for Ni(II), MLX, MLXH, MLXH<sub>2</sub> for Cu(II) and MLX, MLXH for Zn(II), where L = Glu and X = Met. Only these species are refined due to the restricted pH ranges and the possible active forms of ligands like LH<sub>3</sub><sup>+</sup>, LH<sub>2</sub>, LH and XH<sub>2</sub><sup>+</sup>, XH for Glu and Met, respectively.
2. The values of  $\Delta \log K$  indicate that the ternary species have extra stability compared to their binary species,

may be due to the interactions outside the coordination sphere, such as the formation of hydrogen bonds between the coordinated ligands, charge neutralization, chelate effect, stacking interactions and the electrostatic interaction between non-coordinated charge groups of the ligands.

3. The linear increase in the stabilities of ternary complexes with decreasing dielectric constants is due to the dominance of electrostatic forces.
4. The magnitudes of the stability constants for ternary complexes are affected by the errors in the influential parameters like the concentrations of the ingredients. The order of influence is alkali > acid > ligand.
5. The study also gives an insight into the metal availability/metal transport in biofluids. The ternary complexes are more amenable for "metal transport" because of their extra stability.

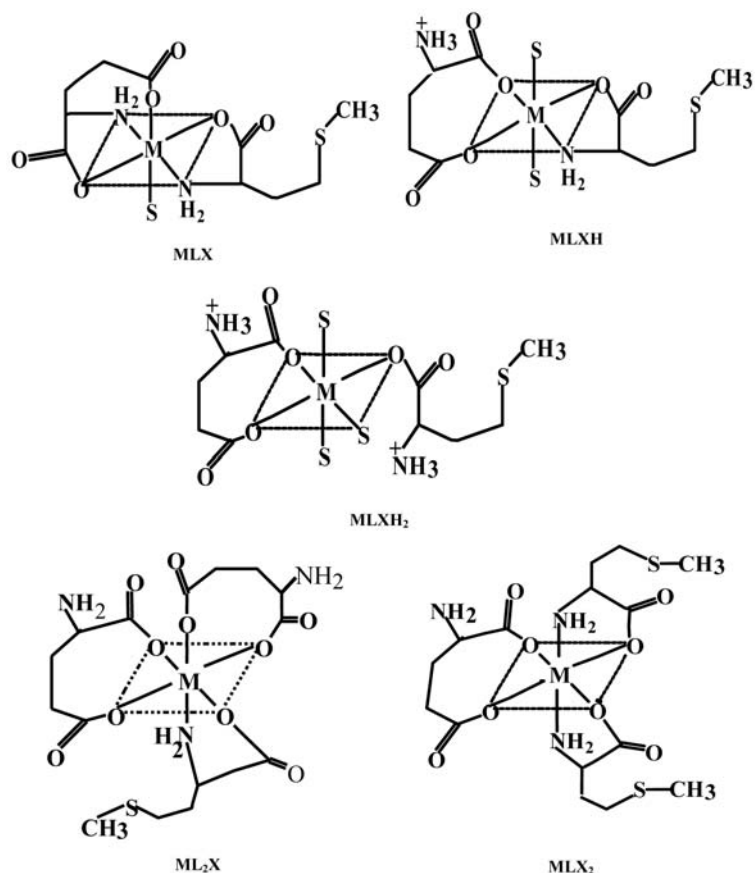


Figure 3: Structures for the ternary complexes of Co(II), Ni(II), Cu(II) and Zn(II) with Glu-Met.

## 5. References

1. A. Satako, T. Abe, T. Yoshioka, S. Kyoritsu, *Busshitsu to Shite* **2003**, 107–136.
2. V. Sauvinet, S. Parrot, N. Benturquia, E. Bravo – Moraton, B. Renaud, L. Denoroy, *Electrophoresis* **2003**, 24, 3187–3196.
3. K. Ajito, C. Han, K. Torimitsu, *Anal. Chem.* **2004**, 76, 2506–2510.
4. E. E. Conn, *Outlines of Biochemistry*, (P. K. Stumpf, Ed.) “4<sup>th</sup> Ed”, Wiley Eastern Ltd., **1976**, 223.
5. D. W. Martin. Jr., *Harper’s Review of Biochemistry*, (P. A. Mayes, V. W. Food Wells, D. W. Granner, Ed.) “20<sup>th</sup> Ed”. Lange Medical Publishers, California, **1985**, 672.
6. M. Noji, K. Saito, Sulphur in plants, A review, **2003**, 135–144.
7. A. L. Lehninger, *Principles of Biochemistry*, “1<sup>st</sup> Ed”. C.B.S. Publishers, New Delhi, India, **1984**, 602.
8. M. P. Latha, V. M. Rao, T. S. Rao, G. N. Rao, *Acta Chim. Slov.* **2007**, 54, 160–165.
9. V. M. Rao, M. P. Latha, T. S. Rao, G. N. Rao, *Serbian Chem. Soc.* In press **2008**.
10. M. P. Latha, V. M. Rao, T. S. Rao, G. N. Rao, *Bull. Chem. Soc. Ethiopia* **2007**, 21, 363–372.
11. V. M. Rao, M. P. Latha, T. S. Rao, G. N. Rao, *Oxidation Commun.* **2007**, 31, 1–13.
12. A. Verma, P. K. S. Chauhan, R. K. Paliwal, *Orien. J. Chem.* **2004**, 20, 365–368.
13. A. Kayali, G. Berthon, *J. Chem.Soc. Dalton* **1980**, 2374–2381.
14. G. Brookes, L. D. Petit, *J. Chem. Soc. Dalton* **1977**, 19, 1918–1924.
15. M. S. Nair, K. Venkatachalapathi, M. Santappa, *J. Chem. Soc. Dalton* **1982**, 291–297.
16. J. Maslowska, L. Chruscinski, *Polyhedron* **1986**, 5, 1135–1138.
17. R. Dodke, F. Khan, *J. Indian Chem. Soc.* **1993**, 70, 14–18.
18. G. C. Cotzias, *Eviron Health-Proc, Univ. Mo. Annu. Conf.* **1967**, 5.
19. G. Gran, *Analyst.* **1952**, 77, 661; G. Gran, *Anal. Chim. Acta* **1988**, 206, 111–123.
20. R. S. Rao, G. N. Rao, *Computer applications in Chemistry*, Himalaya publishing house, Mumbai, **2005**, 302–309.
21. M. S. Babu, G. N. Rao, K. V. Ramana, M. S. P. Rao, *Indian J. Chem.* **2001**, 40A, 1334–1338.
22. G. N. Rao, *Complex equilibria of some biologically important metal ions in aquo-organic media*, Ph. D. Thesis, Andhra



- University, Visakhapatnam, India **1989**.
23. N. Padmaja, M. S. Babu, G. N. Rao, R. S. Rao, K.V. Ramana, *Polyhedron* **1990**, *9*, 2497–2506.
24. P. Gans, A. Sabatini, A. Vacca, *Inorg. Chim. Acta* **1976**, *18*, 237–239.
25. M. Born, *Z Phys.* **1920**, *1*, 45–48.
26. R. Griesser, H. Sigel, *Inorg. Chem.* **1970**, *9*, 1238–1243.
27. R. Griesser, H. Sigel, *Inorg. Chem.* **1971**, *10*, 2229–2232.
28. R. Griesser, H. Sigel, *Inorg. Chem.* **1974**, *13*, 462–465.
29. H. Sigel, P.R. Huber, R. Greisser, B. Prijs, *Inorg. Chem.* **1974**, *12*, 1198–1200.
30. S. Kida, *Bull. Chem. Soc. Japan* **1956**, *29*, 805–811.
31. R. B. Martin, R. Prados, *J. Inorg. Nucl. Chem.* **1974**, *36*, 1665–1670.
32. H. Sigel, *Angew. Chem. Int. Ed. Engl.* **1975**, *14*, 394–402.
33. H. Sigel, *Chimia* **1967**, *21*, 489–500.
34. R. Griesser, H. Sigel, *Helv. Chim. Acta* **1967**, *50*, 1842–1845.
35. T. Sakurai, O. Yamauchi, A. Nakahara, *Bull. Chem. Soc. Japan* **1976**, *49*, 169–173.
36. T. Sakurai, O. Yamauchi, A. Nakahara, *Bull. Chem. Soc. Japan* **1977**, *50*, 1776–1779.

## Povzetek

S potenciometrično (pH) titracijo smo raziskovali tvorbo ternarnih kompleksov Co(II), Ni(II), Cu(II) in Zn(II) ionov z L-glutaminsko kislino and L-metioninom v mešanicah 1,2-propandiola in vode v širokem območju sestave topila (0–60 vol. %) pri konstantni ionski moči  $0.16 \text{ mol dm}^{-3}$  pri 303 K.

Titracije smo izvajali tako, da smo raztopinam z različno relativno koncentracijo kovinskih ionov (M) glede na L-glutaminsko kislino (L) in L-metionin (X) (M: L: X = 1:2:2, 1:2:4, 1:4:2) dodajali 0.4 M NaOH. S pomočjo programa MINIQUAD75 smo eksperimentalnim titracijskim krivuljam prilagodili različne modelne funkcije. Najboljše ujemanje smo ugotovili ob predpostavki naslednjih kompleksov: MLX in  $\text{MLX}_2$  za Co(II); MLX,  $\text{ML}_2\text{X}$  in MLXH za Ni(II); MLX, MLXH in  $\text{MLXH}_2$  za Cu(II); MLX in MLXH za Zn(II).